

EYE-LENS WEIGHT OF THE BULLFROG (*RANA CATESBEIANA*) RELATED TO LARVAL DEVELOPMENT, TRANSFORMATION, AND AGE OF ADULTS¹

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ABSTRACT

Eye-lens growth in larval, transforming, and adult bullfrogs was recorded, and the use of the lens-weight to age adults was examined. Over 500 animals in different developmental stages were captured from ponds in Wood County, Ohio. Using standard techniques, the lenses were removed, fixed, dried, and weighed. The animals also were weighed, measured, and internally sexed. Lens-weight in conjunction with developmental stage allowed separation of cohort populations (those animals from different spawning periods). Adults collected immediately after spring emergence, whose age had been estimated by the pterygoid-bone-ageing method, could be separated into annual classes. The eye-lens weight increased with age, although the rate of increase varied with the developmental stage of the animal. Mean lens-weights were significantly different for each developmental stage in the bullfrog life history, yet considerable overlap and individual variation occurred. The eye-lens growth of poorly-fed laboratory animals continued at a rate similar to that of animals in the wild.

The purposes of this study were 1) to determine the eye-lens growth pattern throughout the developmental stages of the bullfrog, 2) to evaluate the eye-lens weight as a criterion of separating tadpoles of cohort populations and adult annual classes, and 3) to determine the influence of underfeeding on eye-lens growth. Since its introduction by Lord (1959), the use of the eye-lens weight to age animals has been tested by many investigators on a variety of mammals and birds. The lens-weight ageing technique has been more successful in mammals than birds, presumably due to the initial rapid growth in birds (Friend, 1967a). However, the method generally has permitted reliable ageing in early life: separation of individuals of initial litters from those of subsequent litters, juveniles from adults, and annual classes. The technique has had limited application to poikilothermic animals. It was used successfully by Carlton and Jackson (1968) and Burkett and Jackson (1971) to separate annual age-groups of carp and freshwater drum, respectively, but unsuccessfully by Schroeder and Baskett (1968) to separate annual age-classes in summer-collected bullfrogs.

MATERIALS AND METHODS

Bullfrog tadpoles and adults were collected from July, 1970 to June, 1971 (except between December and March) from ponds in the Bowling Green State University's Steidtmann Wildlife Sanctuary and nearby Portage River, Wood County, Ohio (located seven miles south of Bowling Green, at 83°39'20"W longitude and 41°18'20"N latitude). Additional adults were collected immediately after spring emergence in March and April, 1972 from the Sanctuary and aged using the pterygoid-bone method (Schroeder and Baskett, 1968).

The total number of animals caught each month usually exceeded 50. Immediately following collection, animals were etherized and fixed in 10% formalin from one to four weeks, a time interval found by Friend (1967b) to have little effect on the lens weight of rats. Both lenses were removed, oven dried for 48 hours at 120°C, and weighed to the nearest 0.01 mg, (with those less than 0.1 mg not recorded).

To determine the effects of insufficient food on the growth of the lens, tadpoles from two collections (October, 1970 and April, 1971) were kept for two to four weeks under laboratory conditions of 21°C air and water temperatures and constant light (Bruggers, 1971). A small amount of *Elodea* was added to the twenty-gallon aquarium at the onset of each experimental period. It was not replaced as consumed, resulting in a condition of underfeeding. The laboratory animals were sacrificed on or near the date of a wild collection.

All animals were weighed (wet weight, prior to lens removal) to the nearest 0.01 gram, and standard measurements recorded: body length, foreleg length, and hind leg length for

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adults and body, total length, hind and front leg length (when present) for tadpoles. The animals were separated into four developmental groups: A) tadpoles, B) tadpoles with hind legs, C) tadpoles with forelegs and tail (metamorphosing tadpoles), and D) frogs (which were further separated into those with body lengths less than the greater than 10 cm).

RESULTS AND DISCUSSION

General growth and development. The eye-lens weight increased with age, although the rate of increase varied with the developmental stage of the animals, being greatest at the time of metamorphosis (fig. 1). The rate of lens growth was

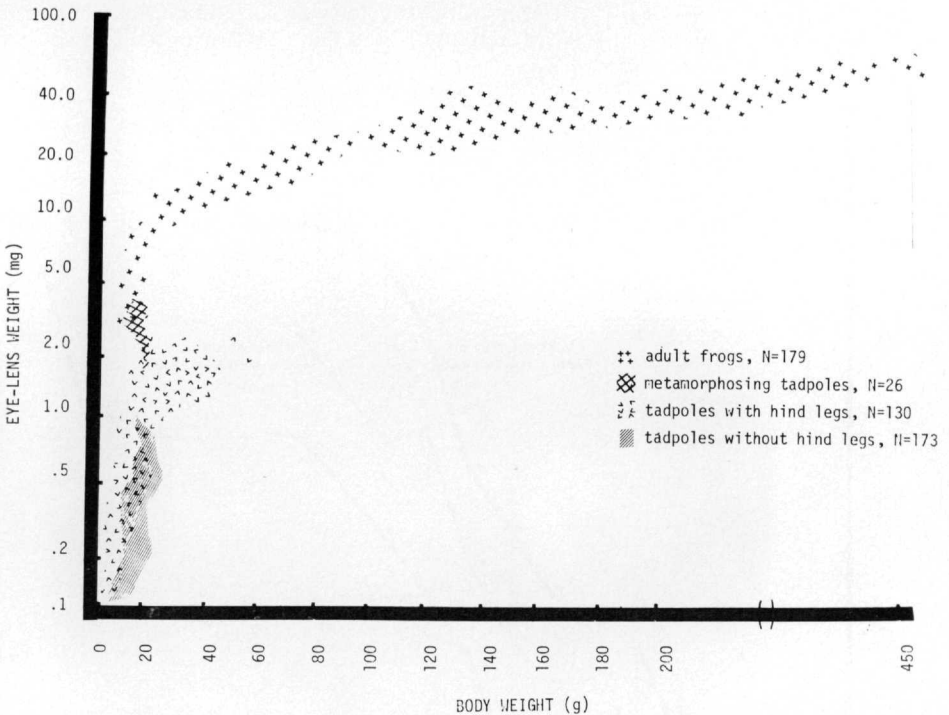


FIGURE 1. Comparison of eye-lens weight and body length for developmental groups of the bullfrog (*Rana catesbeiana*). Shaded areas encompass all points (except where indicated) of a scatter diagram.

slow during early tadpole development; most animals with body weights and lengths less than 4.00 g and 2.7 cm had eye-lens weights less than 0.1 mg. A rapid increase in lens-weight started approximately at the time of hind leg development and continued through metamorphosis into the immature frog stage. Lens growth of adults was slower but continuous. Friend (1967b) also observed a continuous increase in eye-lens weight with age in rats and reported it characteristic of several other species.

The average lens-weights for each morphologic group were significantly different from each other ($p < .001$) using Student's *t*-test, but no significant difference was found between lens-weights of male and female frogs ($p < .05$). Both eye-lens weight and body growth and development patterns for two years were extrapolated from one year's data (fig. 2).

Two major spawning efforts occurred during 1970: one from late May to early June (cohort Population I), and one in late July (cohort Population II). Popula-

tion I tadpoles, hatched in May and June, had body lengths of 2.60 cm by the end of July, growth similar to that observed by Willis, *et al.* (1956), and were the only tadpoles observed without hind legs at that time. By November the mean lens-weight and body length for these group A tadpoles was 0.27 mg and 3.24 cm, respectively.

The spring sample of group A tadpoles indicated only slight increases in body weight and length during the winter, but a mean lens-weight increase from 0.27 mg to 0.41 mg. This apparent continuous winter growth of the lens was unexpected but may substantiate the observations of lens growth of underfed frogs discussed in a later section. Alternatively, the lens growth may have occurred between spring emergence (14–15 March 1970) and the first spring collection, two weeks later.

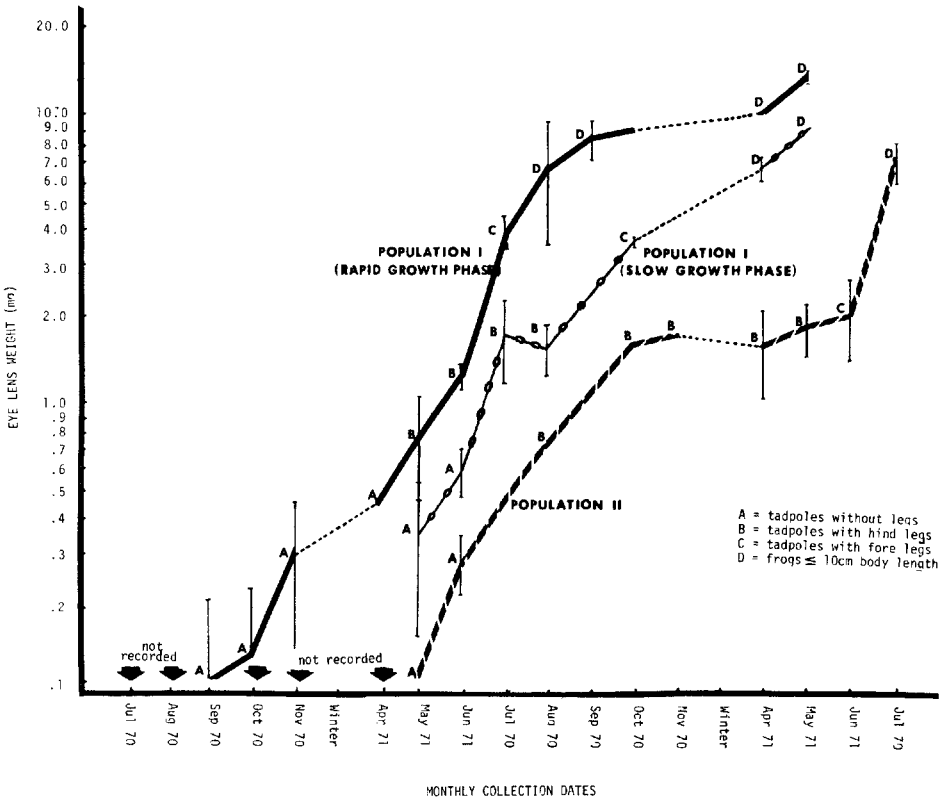


FIGURE 2. Comparison of eye-lens weights during the development of two cohort populations of bullfrogs (*Rana catesbeiana*). Measurements are given as means and standard deviations. Curves are extrapolations derived from one year of sampling for two-year growth patterns.

Tadpoles collected in May, 1971 indicated the possible occurrence of differential growth within Population I, as some animals had developed hind legs (and subsequently metamorphosed in July and August, Rapid Growth Phase), while other animals were without hind legs (and did not metamorphose until October, Slow Growth Phase; fig. 2). George (1940) and Raney and Ingram (1941) also reported larval developmental variation, and related it to water temperature and nutritional habits. Microhabitat differences such as vegetation and crowding also may be important.

Tadpoles hatched in late July or August (cohort Population II) developed more slowly than those hatched in May (cohort Population I). The former spent the next summer as group B tadpoles, and probably metamorphosed the following June, slightly less than two years after hatching. (In June, 1971 many animals, apparently from a late-summer, 1969 hatching, had developed forelegs and were in various stages of resorbing their tails.) Similar variation in duration of larval stages was observed in summer, 1971 and spring, 1972.

The length of time spent in the larval stage varies with geographical locus but generally is longer in the north (Willis, *et al.* 1956). In the present study metamorphosis occurred approximately one year after hatching for animals hatched in early summer and following the second winter for those hatched later in the summer.

In animals of both Population I and Population II the mean body-weight decreased by as much as one-third (also characteristic of *R. pipiens* at metamorphosis; Adolph, 1931), but the mean lens-weight increased more than three-fold by metamorphosis. A further 60-90 percent lens-weight increase was apparent in immature frogs two weeks after metamorphosis. The body weight loss probably is related to the animal's rapid physiological reorganization at metamorphosis, as in approximately a two-week period, lung development, mouth transformation, and digestive tract shortening occur (George, 1940). Lens development at metamorphosis is accompanied by a general reorganization of the eye with transformation to a more terrestrial existence. Eyelids, eye gland, and lacrimal ducts develop; the double cornea of the larva arches and fuses; and the

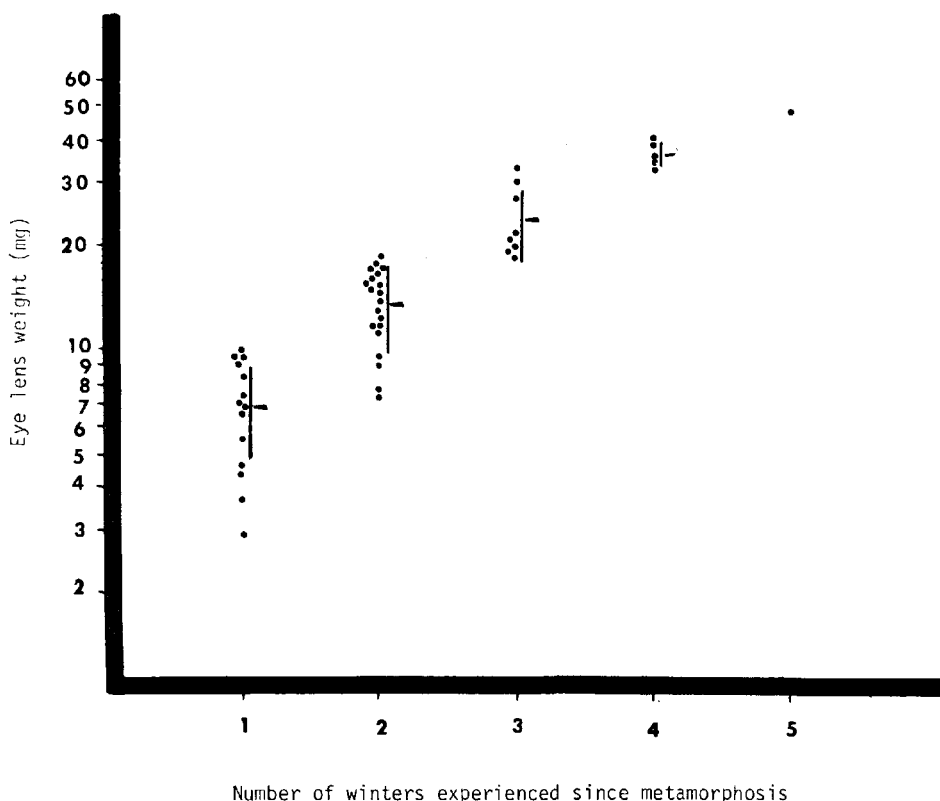


FIGURE 3. Eye-lens weights of 48 adult bullfrogs (*Rana catesbeiana*) collected in March and April, 1972 and aged by the pterygoid-bone method. Means=one standard deviation indicated.

lens migrates closer to the retina, causing a slight increase in farsightedness (Noble, 1954).

Adult ageing. To evaluate the eye-lens weight as a method to separate annual classes in adults, frogs were collected in spring, 1972, immediately after spring emergence, a time when minimal body and lens growth variation should be present. Variation presumably was a major reason for the failure of the lens-ageing technique in the summer-collected frogs of Schroeder and Baskett (1968). Following collection, the animals were aged using the pterygoid-bone method (Schroeder and Baskett, 1968), and the eye-lens removed. Although overlap of individual lens-weights existed between animals of successive annual age-groups, the comparison of mean lens-weights of the successive annual age-groups to five years showed statistical significance ($p < .05$; fig. 3).

Because of the occurrence of individual overlap between age-classes, the lens weight does not depict age precisely. However, establishment of the relationship between lens-weight and an alternate ageing method, such as use of the pterygoid-bone, for a local frog population might permit subsequent age estimates using eye-lens weights that were accurate within one annual class.

Laboratory results. Group A tadpoles of the 16 October 1970 sample kept in the laboratory until 9 November 1970 did not show growth and development comparable to that of animals caught on 23 November 1970, except in lens weight: 0.33 mg for lab animals to 0.27 mg for wild animals. Jackson (1913, cited in Friend and Severinghaus, 1967) stated that the growth of the eye may be somewhat independent of influences that affect the body growth as a whole. Continued lens growth under conditions of underfeeding also was observed in laboratory rats by Friend (1967c), further substantiating the independent nature of lens growth.

Group B laboratory tadpoles from the 5 April 1971 collected metamorphosed in June, 1971 as did the wild animals. However, based on tail reabsorption metamorphosis was between one and two weeks earlier in the laboratory animals, presumably due to the more constant and favorable laboratory temperatures. That the eye-lens continued to develop in these metamorphosing animals despite underfeeding provides further support for independent growth of the lens, perhaps deriving energy from the food stores in the tail.

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